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(54) The use of peptides as medicaments and certain novel peptides.

(57) Certain peptides containing the peptide chain His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp, some of which are novel, can be used as a medicament.

Pharmaceutical compositions are also provided, as are processes for preparing the peptides.

EP 0 044 168 A1

0044168

Title: "The use of peptides as medicaments and certain novel peptides"

THE PRESENT INVENTION relates to the use of peptides of the general formula I

$$R^1\text{-}R^2 \qquad (I)$$

wherein $R^1$ represents His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-
5 10

-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-, and $R^2$ represents OH, the peptide
15 20

chain -Phe-Val-Gln-Trp-Leu or -Met-Asn-Thr or a corresponding peptide
25

chain which is identical with the two last-mentioned peptide chains with the proviso that one or more of the amino acid(s) has/have been omitted, or salts thereof. Compounds of formula I show interesting and surprising pharmacological properties.

Glucagon, a polypeptide hormone consisting of 29 amino acids, is known to possess several pharmacological effects. The use of glucagon for the treatment of hypoglycemia is based upon its metabolic effects. Furthermore, glucagon exerts a spasmolytic effect on smooth muscle and an inhibitory effect on gastric acid secretion. It has now, surprisingly, been found that compounds of formula I as to quantity possess a similar spasmolytic effect and a similar inhibitory effect on gastric acid secretion as that of glucagon, although compounds of formula I show no or minor, negligible metabolic effect. Hence, compounds of formula I are considered superior to glucagon when only a spasmolytic effect or an inhibition of gastric acid secretion is desired.

Glucagon $_{1\text{-}21}$, glucagon $_{\text{-}26}$ and des (22-26) glucagon have for instance been found t have almost the sam potency as glucagon as regards inhibitory effect on the amplitude of the contractions of the electrically

stimulated guinea pig ileum in vitro. $10^{-5}$M glucagon caused 83 ± 4% ($\bar{X}$ ± sd, N = 3) inhibition compared to 78 ± 5% for glucagon $_{1-21}$. The effects of $10^{-6}$M was 50 ± 3% and 52 ± 5%, respectively, and of $10^{-7}$M: 27 ± 3% for either peptide.

Furthermore, glucagon $_{1-21}$ has almost the same potency as glucagon with respect to reducing effect on intestinal motility in rabbits in vivo. 100 to 200 µg glucagon and 77 to 154 µg glucagon $_{1-21}$ administered intravenously as a bolus to anaesthetised rabbits of 2.5 to 3.0 kg body weight caused an inhibition of intestinal motility beginning 1 minute after the administration and lasting for about 10 minutes.

The metabolic effects of glucagon $_{1-21}$, glucagon $_{1-26}$ and des(22-26) glucagon, as exemplified by their lipolytic effect on rat free fat cells in vitro and their effect on the activation of the adenylate cyclase in vitro, are negligible compared with the metabolic effects of glucagon. No metabolic effects have been found after administration to rats in vivo.

It has been shown that glucagon releases insulin from the isolated perfused rat pancreas but glucagon $_{1-21}$ has no such effect when it is infused to the same concentration as glucagon. Furthermore, glucagon $_{1-21}$ - contrary to glucagon - does not cause hyperglycemia or release insulin in vivo in rats.

In cats with chronic gastric fistulas glucagon $_{1-21}$ as well as glucagon inhibit pentagastrin stimulated gastric acid secretion. 1 µg/kg pentagastrin subcutaneously administered to gastric fistula cats caused an increase in gastric acid secretion of 856 ± 71 µEq (Eq designated equivalent) acid ($\bar{X}$ ± S.E.M., N = 18). When 2 µg/kg glucagon $_{1-21}$ was administered subcutaneously at the same time as 1 ug/kg pentagastrin the increase in acid output was only 417 ± 104 µEq acid (N = 6).

Glucagon$_{1-21}$ and glucagon are almost equipotent as regards relaxing effect on a submaximally contracted rabbit gall bladder preparation in vitro, and both compounds cause an increase in gall flow in rats in vivo. When a gall bladder strip was contracted with 0.1 µg/ml cholecystochinin octapeptide $10^{-6}$M glucag n caused 39% relaxation and $10^{-6}$ M glucagon $_{1-21}$ caused

41% relaxation. The $ED_{50}$ value was for both peptides $2.7 \times 10^{-6}$M. Therefore, compounds of formula I may have a potential utility in the treatment of biliary tract and - because of their general spasmolytic properties - possibly

urinary calculi patients. As regards this utility, the fact that compounds of formula I have no or minor, negligible metabolic effect must be a considerable advantage.

Hence, a compound of formula I or a salt thereof may be used as a therapeuticum or a diagnosticum. The indication areas for use of the compounds of formula I and salts thereof in therapy will be, for example, biliary tract and urinary tract calculi, spasms in the digestive system and gastro-duodenal ulcers. The indication areas for use of the compounds of formula I and salts thereof for diagnostic purposes will be investigational techniques such as radiology (X-ray examination), endoscopy (direct observation of the gastro-intestinal tract) and hysterosalphingographia.

Compounds of formula I are converted into pharmaceutical preparations and administered, preferably to humans, in analogy with known methods.

Compounds of formula I and salts thereof can, as diagnosticum, be used in analogy with the use of glucagon for the same purpose. Compounds of formula I and salts thereof can be administered intraveneously, intramuscularly or subcutaneously at dosages in the range of from about 1 to 1000 µg/kg body weight, preferably from about 10 to 100 µg/kg body weight, although a lower or higher dosage may be administered. The required dosage will depend on the severity of the condition of the patient and the duration of treatment. A higher dosage may be used for biliary tract and urinary tract calculi patients and gastro-duodenal ulcer patients and, in these cases, multiple dosages of the compounds may be administered, for example, parenterally (for example as a continuous infusion) or by the nasal or rectal route.

Compounds of formula I may possibly be administered orally, e.g. by the use of special additives.

For the purpose of parenteral administration, compounds of formula I are dissolved in destilled water and the pH-value is adjusted to about 6 to 8. In order to facilitate the lyophilization process resulting in a suitable product lactose could be added to the solution. The solution is sterile filtered and filled in vials.

Thereafter, the solutions are lyophilized and the vials are sealed under aseptic conditions.

For the purpose of nasal administration a solution in a nasal spraying device or nebulisator is used. The compounds of formula I are dissolved in destilled water, the pH-value is adjusted to about 6 to 8 by adding sodium phosphate and citric acid as buffer. Sodium chlorine, sorbitol and glycerol are used to obtain an isotonic solution with a suitable viscosity. The solution is administered by the use of a suitable nebulisator or plast spray. The solution may be preserved by the use of known preservatives and a known surfactant may be added.

For the purpose of nasal administration by the use of dose aerosol spray the peptides are mixed with suitable constituents and a mixture of halogencarbons, i.e. monofluorotrichloromethane, difluorodichloromethane and tetrafluorodichloroaethane, in order to obtain a mixture with a vapour pressure procducing a well defined single dose when the mixture is administered by the use of a dose aerosol spray.

The compounds of formula I are preferably used by nasal administration in a dosage range between about 0.1 and 100 µg/kg body weight, preferably between 1 and 10 µg/kg body weight, per single dose. This dose could be administered several times per day.

For the purpose of rectal administration suppositories are produced by admixing compounds of formula I, with an inactive constituent such as cocoa butter or with a base such as Polysorbate 85, propylene glycol monostearate and white bee's wax.

Compounds of formula I and salts thereof can be prepared by methods which are generally known in peptide synthesis. Briefly, compounds of formula I can be built up from a protected glucagon fragment, e.g. protected glucagon$_{1-15}$, and a protected peptide containing the remaining amino acids of the desired compound of formula I. The preparation of protected glucagon$_{1-15}$ is described in Res.Discl. 1979, 247. Peptides containing more than amino acids Nos. 16 - 21 in glucagon can be built up from a protected glucagon fragment, e.g. protected glucagon $_{16-21}$, and a

protected peptide containing the remaining amino acids. The use of suitable protecting groups and activations during the peptide synthesis is known t the skilled art worker. It is desired to use protecting groups which can easily be removed.

Thus, glucagon$_{1-21}$, glucagon$_{1-26}$ and des (22-26)-glucagon can be prepared by coupling the protected glucagon fragment:

Adoc-His(Adoc)-Ser(Bu$^t$)-Gln-Gly-Thr(Bu$^t$)-Phe-Thr(Bu$^t$)-Ser(Bu$^t$)-
5
-Asp(OBu$^t$)-Tyr(Bu$^t$)-Ser(Bu$^t$)-Lys(Boc)-Tyr(Bu$^t$)-Leu-Asp(OBu$^t$)-OH (II)
10 15

with the protected glucagon fragments:

H-Ser(Bu$^t$)-Arg(HCl)-Arg(HCl)-Ala-Gln-Asp(OBu$^t$)-OBu$^t$ (III)
20

H-Ser(Bu$^t$)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu$^t$)-Phe-Val-Gln-Trp-
20 25
(IV)
-Leu-OBu$^t$

or H-Ser(Bu$^t$)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu$^t$)-Met-Asn-Thr(Bu$^t$)-
20
(V)
-OBu$^t$

respectively, by the mixed anhydrid method using isobutyl chlorcformate. The fully protected peptides so obtained can be deprotected under acid conditions, e.g. by treatment with trifluoroacetic acid containing 10% 1,2--ethanedithiol. The crude peptides can be purified by ion-exchange chromatography, e.g. QAE-Sephadex A-25, followed by a desalting procedure, e.g. gel-filtration on Sephadex G-25. The purified peptides can be isolated by lyophilization. The intermediate protected glucagon fragments IV and V can be prepared by coupling, using the mixed anhydride procedure, the protected glucagon fragment:

Bpoc-Ser(Bu$^t$)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp(OBu$^t$)-OH (VI)
20

with the protected glucagon fragments:

H-Phe-Val-Gln-Trp-Leu-OBu$^t$ (VII)

or H-Met-Asn-Thr(Bu$^t$)-OBu$^t$ (VIII)

respectively, whereupon the N-terminal Bpoc group can be removed selectively under mild acid conditions, e.g. by treatment with HCl (0.2N) in methanol/N,N-dimethylformamide.

The protected peptide fragments III, VI, VII and VIII were synthesized by stepwise chain elongation applying conventional procedures such as the active ester or mixed anhydrid methods for coupling.

Peptides of formula I, wherein $R^2$ represents the peptide chain -Phe--Val-Gln-Trp-Leu or -Met-Asn-Thr in which one or more amino acid(s) has/have been omitted, can be prepared in a similar manner as described above with the exception that one or more of the amino acid(s) in question has/have been omitted in the protected peptide fragments VII and VIII.

A process for preparing glucagon$_{1-21}$ has been described in J.Biol.Chem. 247, 2133, by digesting porcine, bovine or sheep glucagon with carboxypeptidase A. Glucagon$_{1-26}$ is known from Metabolism 25, Suppl. 1, 1315.

A preferred subclass of compounds of formula I is compounds wherein the amino acid sequence is identical with a continuous part of the amino acid sequence of glucagon. As examples of specific compounds, within this class of compounds, compounds of formula I, wherein $R^2$ is Phe, Val, Gln, Trp, Leu, Met, Asn or Thr, can be mentioned. A preferred compound of formula I is glucagon$_{1-21}$, because it shows superior pharmacological properties and because it can easily be obtained, e.g. from natural glucagon.

Furthermore, the present invention relates to novel compounds of the general formula I'

$$R^1\text{-}R'^2$$

wherein $R^1$ is as defined above, and $R'^2$ has the same meaning as $R^2$, provided that $R'^2$ does not represent -Phe-Val-Gln-Trp-Leu or OH, or a salt thereof.

Briefly, compounds of formula I' may be prepared by treating a compound of the general formula

$$R^3-R^4-OBu^t \qquad \text{(IX)}$$

wherein $R^3$ represents Adoc-His(Adoc)-Ser($Bu^t$)-Gln-Gly-Thr($Bu^t$)-
-Phe-Thr($Bu^t$)-Ser($Bu^t$)-Asp($OBu^t$)-Tyr($Bu^t$)-Ser($Bu^t$)-Lys(Boc)-
-Tyr($Bu^t$)-Leu-Asp($OBu^t$)-Ser($Bu^t$)-Arg(HX)-Arg(HX)-Ala-Gln-
Asp($OBu^t$)-,

$R^4$ represents the peptide moiety -Phe-Val-Gln-Trp-Leu-
from which one or more of the amino acid(s) has/have been omitted, the peptide moiety
-Met-Asn-Thr($Bu^t$)- or corresponding peptide moieties which are identical with said moiety with the proviso that one or more of the amino acid(s) has/have been omitted, and X represents chlorine or bromine, with an acid such as trifluoroacetic acid.

As examples of salts of compounds of formula I, for example sodium, potassium, magnesium, calcium and zink salts and acid addition salts with organic or inorganic acids such as formic acid, methansulfonic acid, hydrochloric acid and sulphuric acid can be mentioned. Preferred salts of compounds of formula I are physiologically and pharmaceutically acceptable salts.

The present invention also relates to a pharmaceutical composition comprising a compound of formula I or a salt thereof and one or more pharmaceutically acceptable carrier(s), diluent(s) preferably water, and/or excipient(s). As examples of such carriers conventional preservatives, e.g. methyl or propyl p-hydroxybenz ate, and sodium chloride can be mentioned.

Any novel feature or combination of features described herein is considered essential.

The nomenclature used herein complies with that stated in J. Biol.Chem. 247, 977, and Biochem. J. 104, 17. However, for the sake of brevity, glucagon-(1-21)-heneicosapeptide herein has been designated glucagon$_{1-21}$, glucagon-(1-26)-hexacosapeptide has been designated glucagon$_{1-26}$ and des-pentapeptide-(22-26)-glucagon has been designated des(22-26)glucagon. Bpoc represents 1-(biphenyl-4-yl)-1-methylethoxy-carbonyl, Adoc represents 1-adamantyloxycarbonyl, Bu$^t$ represents tertiary butyl, and Boc represents tert-butyloxycarbonyl.

The following examples which, however, are not considered to be limiting are presented to illustrate the invention.

Example 1

des(22-26)glucagon

1 g of Adoc-His(Adoc)-Ser($Bu^t$)-Gln-Gly-Thr($Bu^t$)-Phe-Thr($Bu^t$)-
-Ser($Bu^t$)-Asp($OBu^t$)-Tyr($Bu^t$)-Ser($Bu^t$)-Lys(Boc)-Tyr($Bu^t$)-Leu-
-Asp($OBu^t$)-Ser($Bu^t$)-Arg(HBr)-Arg(HBr)-Ala-Gln-Asp($OBu^t$)-Met-
-Asn-Thr($Bu^t$)-$OBu^t$ is dissolved in 25 ml of trifluoroacetic acid containing 10% 1,2-ethanedithiol and the reaction mixture is left at 15° C for 3 hours. Thereafter, 200 ml of tetrahydrofuran is added slowly and the precipitate is isolated, washed with tetrahydrofuran and dried in vacuo. The resulting product may be purified by ion-exchange chromatography on QAE Sephadex A-25 and desalted by gel-filtration on Sephadex G-25.

Example 2

A preparation for parenteral administration containing 1 mg of glucagon$_{1-21}$ per ml may be prepared as follows:

1 g of glucagon$_{1-21}$ and 99 g of lactose are dissolved in 1 litre of distilled water and the pH-value is adjusted to 7.0. The solution is thereafter sterile filtered. The sterile solution is filled in 10 ml vials in such a way that each vial contains 1.0 ml of the solution. Thereafter, the solutions are lyophilized and the vials are sealed under aseptic conditions.

The preparation in any of the vials is to be dissolved in 1.0 ml of sterile, isotonic water before administration.

**Example 3**

A preparation for parenteral administration containing 10 mg of glucagon$_{1-21}$ per ml may be prepared as follows:

10 g of glucagon$_{1-21}$ and 90 g of lactose are dissolved in 1 litre of distilled water and the solution is prepared analogously to the method described in Example 2.

Example 4

Rectal suppositories are prepared by admixing 1 mg of glucagon$_{1-21}$ with 4 g of cocoa butter.

Example 5

A nasal plast spray may be prepared as follows:

0.5 g of glucagon$_{1-21}$ is dissolved in about 95 ml of 0.01 M phosphate buffer (pH-value: 7.4) which is made isotonic by the addition of glycerol. The solution is preserved by the addition of 0.01% benzalkonium chloride and 0.05% EDTA whereafter 0.5% polyoxysorbate is added. An isotonic phosphate buffer is added in order to give a resulting volume of 100 ml and the solution is sterile filtered. 15 ml of said solution is filed in a plast spray giving 0.5 mg of glucagon$_{1-21}$, when activated.

Experiment A: Spasmolytic Effect

One male rabbit weighing 2.56 kg was anaesthetized with nembutal after an overnight fast. The position of the balloon used for measurement of intestinal motility was 1 meter from pyrolus in the jejunum. The motility was registered before and after intraveneous administration of 77 ug glucagon$_{1-21}$ in 1 ml 0.9% saline containing 0.1% human serum albumin. The effect obtained was nearly complete atonia of the intestine. The onset of effect was 1 minute after the administration and the duration of effect was 11 minutes.

Experiment B: Spasmolytic Effect

A male rabbit weighing 2.32 kg was treated as described in Experiment A with the following dosages:

77 µg glucagon$_{1-21}$ in 1 ml of the solution stated in Experiment A intraveneously caused no detectable spasmolytic effect.

154 µg glucagon$_{1-21}$ in 1 ml of the solution in Experiment A intraveneously had a questionable effect. 308 µg glucagon$_{1-21}$ in 1 ml of the above solution had a distinct spasmolytic effect causing nearly complet atonia. The onset of the effect was 2½ minute and the duration of the effect was 6 minutes.

For comparison glucagon was administered to the same rabbit. 200 ug glucagon intravenously had no detectable effect, however, 400 µg gave a distinct effect comparable to the effect caused by 308 µg glucagon$_{1-21}$.

Experiment C: Gastric Acid Inhibitory Effect

In a male cat weighing approx. 4.5 kg equipped with a cronic gastric fistula the gastric acid secretion was stimulated with 4.5 µg pentagastrin (Peptavlon$^{(R)}$) in a volume of 1 ml 0.9% saline containing 0.1% human serum albium subcutaneously in the neck. In 8 experiments 1 ml placebo (0.9% saline with 0.1% human serum albumin) was administered subcutaneously through another cannula in the neck at the same time as the

administration of pentagastrin. In 2 experiments 9 µg f glucagon$_{1-21}$ in 1 ml of the above solution was administered simultaneously with the administration of pentagastrin. Gastric acid secretion was collected over periods of 15 minutes and titrated with 0.01N NaOH. The increase in acid secretion after the administration of petagastrin was calculated as µEq acid excreted over 1½ hrs. after the administration subtracting the basal acid secretion before administration of pentagastrin. After administration of 4.5 µg pentagastrin plus placebo the increase in gastric acid secretion was 729 ± 89 µEq acid ($\bar{X}$ ± S.E.M., N = 8). 4.5 µg pentagastrin + 9 µg glucagon$_{1-21}$ caused an increase in acid secretion of 238 µEq in one experiment and 231 µEq in another experiment.

Experiment D: Effect on Bile Flow

In rabbits with catheters in the bile duct the administration of glucagon and glucagon$_{1-21}$ caused a decrease in gall flow immediately after the administration, probably reflecting a decrease in the tonus of the gall bladder. This decrease in flow was followed by an increase in bile flow to quantities higher than before the administration reflecting an increase in production of bile.

One rex rabbit weighing 2.0 kg was equipped with a catheter in the bile duct during nembutal anaesthesia on the day before the experiment. On the day of the experiment the bile was collected for periods of 15 minutes.

The results obtained appear from the following table:

| Sampling periods, minutes | 0-15 | 15-30 | 30-45 | 45-60 | 60-75 | 75-90 | 90-105 | 105-120 | 120-135 | 135-150 |
|---|---|---|---|---|---|---|---|---|---|---|
| Amount of bile, ml | 1.20 | 1.50 | 1.40 | 0.20 | 0.25 | 3.30 | 2.80 | 2.00 | 0.40 | 1.65 |

| Sampling periods, minutes | 150-165 | 165-180 | 180-195 | 195-210 | 210-225 | 225-240 | 240-255 | 255-270 |
|---|---|---|---|---|---|---|---|---|
| Amount f bile, ml | 2.05 | 3.50 | 1.10 | 1.50 | 1.50 | 1.35 | 1.70 | 1.75 |

After 45 minut s 200 µg glucagon was administered subcutaneously in 1 ml of 0.9% saline containing 0.1% human serum albumin. After 120 minutes 154 µg glucagon$_{1-21}$ was administered subcutaneously in 1 ml of the above solution. After 195 minutes the placebo (vide Experiment C) was administered.

Experiment E: Acute Toxicity Study

10 mg glucagon$_{1-21}$ administered intravenously as a bolus to NMRI mice weighing 20 g (i.e. a dose of 500 mg/kg body weight) had no adverse effects. No deaths occurred.

CLAIMS

1. The use, as a medicament or diagnosticum, of a compound of the general formula I

$$R^1-R^2 \qquad (I)$$

wherein $R^1$ represents His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp- and $R^2$ represents OH, the peptide chain -Phe-Val-Gln-Trp-Leu or -Met-Asn-Thr or a corresponding peptide chain which is identical with the two last-mentioned peptide chains, with the proviso that one or more of the amino acid(s) thereof has/have been omitted, or a salt of such a compound.

2. The use according to Claim 1, wherein the compound is used as a spasmolyticum or a gastric acid secretion depressing agent.

3. The use according to Claim 1, wherein the compound is used for treatment of spasms in the digestive system, for treatment of biliary tract and urinary tract calculi and/or for treatment of gastro-duodenal ulcers.

4. The use according to any one of Claims 1 to 3, wherein $R^2$ represents OH, -Phe-Val-Gln-Trp-Leu or -Met-Asn-Thr.

5. The use according to any one of Claims 1 to 4, wherein $R^2$ represents OH.

6. A pharmaceutical composition, which comprises an effective amount of a compound of formula I of Claim 1, or a salt thereof, in association with a suitable physiologically acceptable carrier, diluent and/or excipient.

7. A pharmaceutical composition according to Claim 6, which comprises from 7.5 to 75,000 µg, preferably from 75 to 7500 µg, of a compound of formula I, or salt thereof, per dosage unit.

8. A pharmaceutical composition according to Claim 6 or 7, wherein $R^2$ represents OH, -Phe-Val-Gln-Trp-Leu or -Met-Asn-Thr.

9. A pharmaceutical composition according to Claim 6 or 7, wherein $R^2$ is OH.

10. A novel compound of the general formula I'

$$R^1\text{-}R'^2 \qquad (I')$$

wherein $R^1$ is as defined in Claim 1 and $R'^2$ has the same meaning as $R^2$ as defined in Claim 1, provided that $R'^2$ does not represent -Phe-Val-Gln-Trp-Leu or OH, or a salt of such a compound.

11. A process for the preparation of a compound of the formula I' or a salt thereof, which comprises preparing the same from the parent L-amino acids, whereafter a compound of formula I', if desired, is converted into a salt thereof.

12. A process for the preparation of a compound of the formula I', or a salt thereof, wherein a compound of the general formula

$R^3-R^4-OBu^t$ (IX)

wherein $R^3$ represents Adoc-His(Adoc)-Ser($Bu^t$)-Gln-Gly-Thr($Bu^t$)-Phe-Thr($Bu^t$)-Ser($Bu^t$)-Asp($OBu^t$)-Tyr($Bu^t$)-Ser($Bu^t$)-Lys(Boc)-Tyr($Bu^t$)-Leu-Asp($Obu^t$)-Ser($Bu^t$)-Arg(HX)-Arg(HX)-Ala-Gln-Asp($OBu^t$)-, $R^4$ represents the peptide moiety -Phe-Val-Gln-Trp-Leu- from which one or more of the amino acid(s) has/have been omitted, the peptide moiety -Met-Asn-Thr($Bu^t$)- or corresponding peptide moieties which are identical with said moiety with the proviso that one or more of the amino acid(s) has/have been omitted, and X represents chlorine or bromine, is treated with an acid, such as trifluoroacetic acid, whereafter the resulting compound, if desired, is converted into a salt thereof.

European Patent Office

# EUROPEAN SEARCH REPORT

0044168

Application number
EP 81 30 2978

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.³) |
|---|---|---|---|
| X | THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 247, no. 4, February 25, 1972, I.D. GODLFINE et al. "Glucagon receptors in β-cells. Binding of $^{125}$I-glucagon and activation of adenylate cyclase", pages 1211-1218<br>* Whole article *<br>-- | 1-6, 8-10 | C 07 C 103/52<br>A 61 K 37/28 |
| | US - A - 3 642 763 (E. WUNSCH)<br>* Whole document *<br>---- | 1,2 | |

TECHNICAL FIELDS SEARCHED (Int. Cl.³)

C 07 C 103/52
A 61 K 37/28

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons
&: member of the same patent family, corresponding document

X The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| The Hague | 16-10-1981 | RAJIC |

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